Study on histopathology of nuclear polyhedrosis virus of *Zethenia rufescentaria* Motsch.[1)]

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Abstract This paper reports the histological observation of larvae of *Zethenia rufescentaria* Motsch. after infection by ZrNPV. Histopathologic study revealed that ZrNPV were multiplied within the nuclear of fat body, epidermis cell, midgut cell, tracheal matrix and blood cell. These cells showed obvious cytopathic effects. The nucleus of infected cells underwent swelled. Under electron microscope, virus and polyhedral of ZrNPV were clearly observed in these nucleus of infected cells. The nucleus of susceptible tissues were fulfilled with polyhedra after 70~140 h.
Key words: *Zethenia rufescentaria*, Nuclear polyhedrosis virus, Histopathology

Introduction

Zethenia rufescentaria Motsch. is one of the main pests in larch plantation in Northeast China. In recent years, the pest caused disaster in the forest area of Heilongjiang Province. The needles of larch in some area were been eaten up completely and the growth of larch plantation was seriously affected. The nuclear polyhedrosis virus of *Z. rufescentaria* Motsch (ZrNPV) was obtained from the larvae of *Z. rufescentaria* Motsch. in 1991. ZrNPV was main pathogen of the larvae of *Z. rufescentaria* Motsch. Toxicity measuring indoors and investigation in field proved that ZrNPV had a strong toxicity and could cause epidemic disease of viruses among hosts in a large area of forest quickly. ZrNPV is one of the main measures to control the pest larvae of The mechanism of infecting host tissues and duplicating procedure in cells of ZrNPV had not been reported in the past. The histopathology of ZrNPV was studied in detail with optical and electron microscopes in order to understand the procedures of infection, duplication and destroying host tissues of ZrNPV and make a basis for the reproduction and utilization of ZrNPV.

Materials and methods

The NPV was produced at 4 °C in 1993. Polyhedron was obtained by centrifugal effect. Overwintered pupae of *Z. rufescentaria* Motsch. were selected in larch plantation in Hulin County of Heilongjiang Province in the last ten days of April of 1994. The adults emerged, mated and oviposited in laboratory. After sterilized, the eggs hatched and developed to 2nd-instar larvae feeding on sterilized needles.

The sterilized needles of larch were immersed in polyhedron suspension, and after dried, 2nd-instar larvae of *Z. rufescentaria* Motsch. was fed on them. The samples were selected after 2, 5, 8, 10, 20, 25, 30, 50, 56, 70, 90, 115, 140, 160, 168, 190, 210, 240, 330 and 360 h. of inoculation respectively. The samples were fixed with glutaric aldehyde and Osmium tetroxide, and dehydrated with alcohol, then imbedded with Epon 618. The imbedded tissues were cut by super-thin microtome to half-thin microtome section (observed with optic microscope) and to super-thin microtome section

Results

The tissues infected by ZrNPV

ZrNPV mainly infected the fat cells, epidermis cells, midnut cells, tracheal matrix and blood cells of the larvae of *Z. rufescentaria* Motsch (Yan *et al* 1991, Deng *et al* 1991; Yu *et al* 1993). At first, polyhedrons were formed in fat tissues. Virions began to infect cells after 30 h of inoculation (Fig 1.1～1.2).

Histopathology and duplication of viruses

fat cells

The cells nucleus of fat cell began to swollen after 70 h, and lots of polyhedrons appeared after 90 h. The nucleus was filled with formed polyhedrons after 168 h (Fig. 1. 4). But there was difference in the forming rate of polyhedrons. Some polyhedrons were forming (Fig 1.5). There were formed, forming and free poly-

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hedrons in some nucleus (Fig. 1. 6). Some nucleus were destroyed and filled with polyhedrons (Fig.1.7).

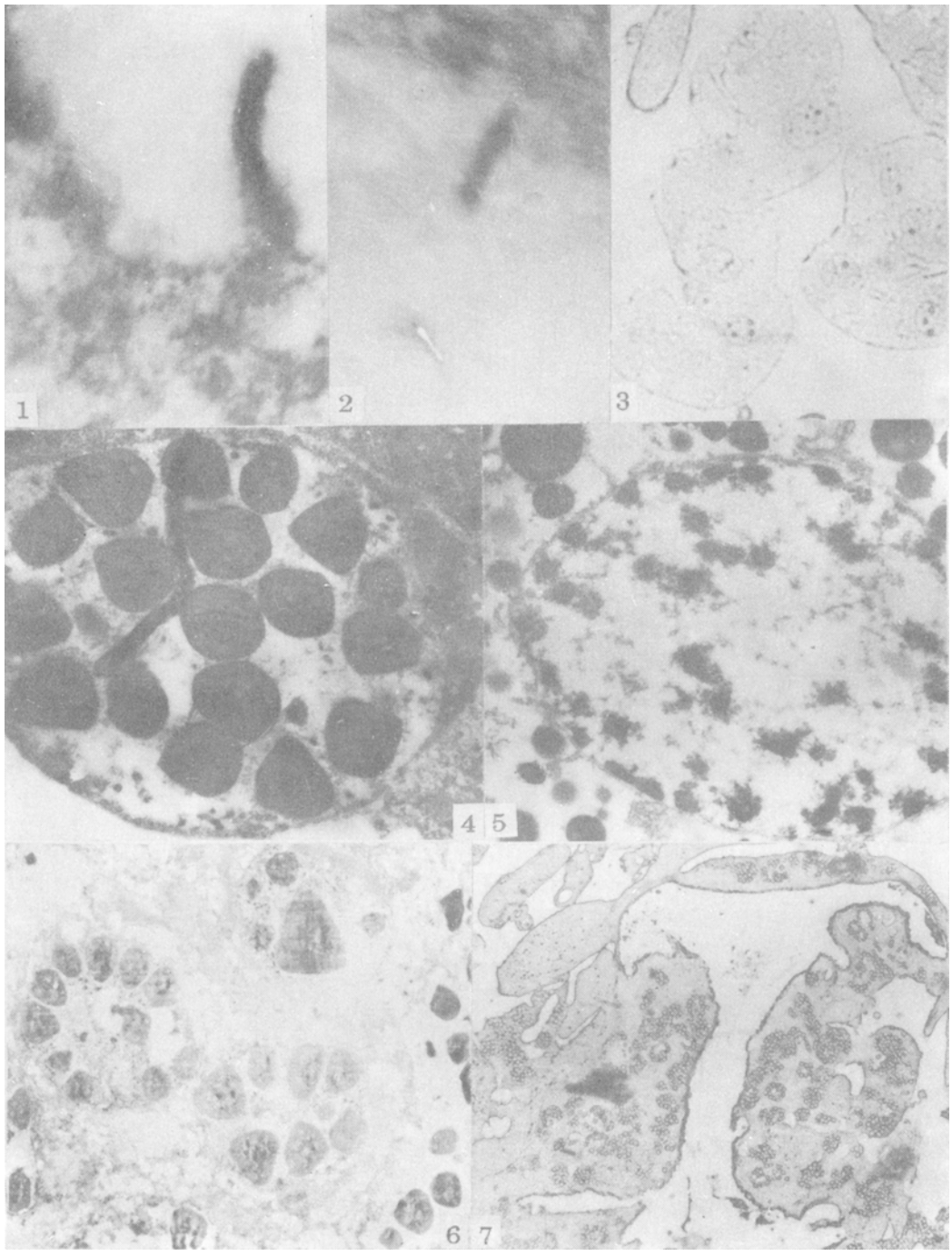

Fig. 1. Histopathology of ZrNPV of *Zethenia rufescentaria* Motsch.

1. Virion was attaching cell memberane after infection for 30 h (X75 000); **2.** Virion had entered cell after infection for 30 h (X114 000); **3.** Polyhedrons were formed in nucleus after 70 h infection (photo of optic microscope); **4.** The nucleus of fat cell was filled with polyhedrons after 168 h infection (X6 000); **5.**Polyhedrons were forming in some fat cell nucleus after infection 168 h (X9 500); **6.** There existed formed polyhedrons, virus cluster entering polyhedron protein and virions in the same cell nucleus of fat cell (X3 500); **7.** .All fat cells were destroyed after 360 h of infection and lots of free polyhedrons among tissues (photo of optic microscope)

epidermis cells

There appeared small number of formed and lots of forming polyhedrons in nucleus after 90 h. of inoculation (Fig. 2.1). The epidermis cells were destroyed by polyhedrons after 168 h, and there were lots of polyhedrons in them (Fig. 2.2). All of the cells were destroyed after 190 h (Fig. 2.3).

Midgut cells

The nucleus of midgut cell enlarged after 70 h, polyhedrons appeared in nucleus after 90 h. The nucleus of midgut cells were destroyed after 240 h. The Destroyed tissues were filled with polyhedrons (Fig. 2.4).

Tracheal matrix

After 70 h of inoculation, the histopathology was changed in tracheal matrix. Nucleus enlarged after 90 h. There existed polyhedrons in nucleus after 140 h, and many polyhedrons were forming (Fig 2.5). All of tracheal matrix cells dropped after 240 h. (Fig. 2.6).

Blood cells

There were lots of polyhedrons in nucleus after 115 h. (Fig. 2.7).

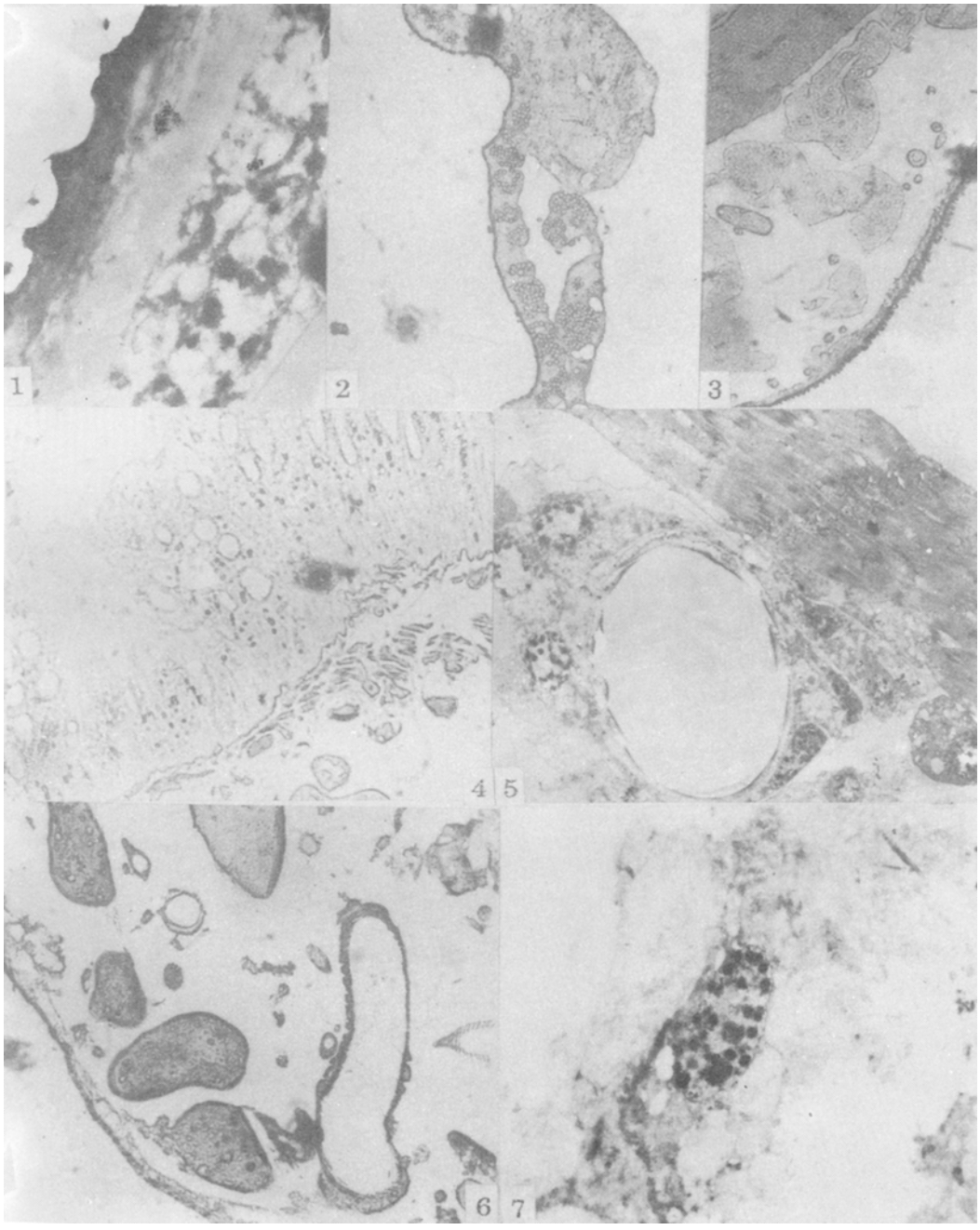

Fig. 2. Histopathology of ZrNPV of *Zethenia rufescentaria* Motsch.

1. A small number of formed and forming polyhedrons in cell nucleus of epidermis cells after 90 h infection (X3000); 2. Epidermis cell nucleus was destroyed completely after 168 h and there were lots of polyhedrons in tissues (photo of optic microscope); 3. The destroyed epidermis cells after 190 h (photo of optic microscope); 4. Midgut cells were destroyed after 240 h. and there were lots of polyhedrons among tissues (optic microscope); 5. Polyhedrons were formed in the nucleus of tracheal cells after 140 h infection(X2000); 6.All of tracheal matrix cells dropped after 240 h. infection (photo of optic microscope); 7.There appeared polyhedrons in blood cell nucleus after 115 h.

Summary and discussion

ZrNPV mainly infects fat cells, tracheal matrix cells, epidermis cells, midgut cells and blood cells.

The virions attacked infected cells after 30 h, nucleus enlarged after 56 h. Polyhedrons began to be formed after 70~140 h. Some nucleus were destroyed after 168 h. All infected tissues were destroyed after 360 h, and insects died.

According to some documents (Huang 1987; Zhang 1988), ZrNPV can infect muscular sheath, nerve sheath and pericardial cells besides the five kinds of cells mentioned above. This situation did not occurred in this study. Further study should be carried out.

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